Project title:	Detection and control of downy mildews on ornamentals
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The results and conclusions in this report are based on an investigation conducted over a three-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Headline

Criteria required for the infection of Impatiens and pansies by downy mildew have been established and several products with good activity against pansy downy mildew have been identified.

Background and expected deliverables

Downy mildew of *Impatiens walleriana* or 'Busy Lizzy', was first reported in the UK by STC in June 2003 causing considerable economic damage to commercial crops and municipal plantings, especially, though not exclusively, in the South of England. STC provided a presumptive identification as *Plasmopara obducens* and, as it was considered unusual and a potential first UK record, the Plant Health & Seeds Inspectorate (PHSI) was notified. The identity of the fungus was subsequently confirmed as *P. obducens.* Emergency statutory action was immediately taken by PHSI under Article 22 of the Plant Health Order 1993 and the downy mildew pathogen on Impatiens was declared notifiable, thus requiring the industry to notify PHSI of any suspect cases of the disease. Where the pathogen was subsequently confirmed, statutory action was taken to destroy the infected plants and to quarantine (for a pre-determined time period) the remaining apparently disease-free stock. If the disease was subsequently found in this stock it was also destroyed. The cost of such crop loss had to be met by the individual grower, as government compensation for crop loss is not available. A cost-benefit analysis of management options for the control of Impatiens downy mildew was undertaken (Jones, 2004) in which it concluded: "the most cost-effective option for government would be for the industry to police itself in the future as regards this disease". This was a view supported by the industry and in May 2005, Defra announced that statutory action would no longer be taken and that the industry would take on responsibility for management of the disease through implementation of an industry code of practice (Good Horticultural Practice (GHP)).

In order for the industry to take responsibility for the overall management of downy mildew, it is necessary for growers to have at their disposal effective techniques for the detection of early (latent) infections of downy mildew, potentially on both seed and young propagation material. They also require access to a range of approved crop protection products with demonstrated activity and crop safety against *Plasmopara obducens, Peronospora violae* and other downy mildew fungi in

ornamental crops such as *Peronospora tabacina* in Nicotiana, *Peronospora parasitica* in Cheiranthus/Erysimum and *Peronospora antirrhini* in Antirrhinum. Any recommended spray programme must also take due regard of the resistance risk with certain pathogen groups e.g. phenylamides and ensure a range of products with different modes of action is available to minimise the selection of tolerant and/or resistant strains in the pathogen population. Baseline sensitivity data would be valuable in this regard to allow shifts in pathogen sensitivity to different fungicides to be monitored over time.

Following the initial 2003 outbreaks, when this project was commissioned, further occurrences of Impatiens downy mildew throughout Europe, including the UK, remained absent or low. This made it very difficult to progress the work with *Plasmopara obducens* as the pathogen involved is an obligate organism and therefore cannot be grown or maintained on agar. In order to ensure progress within the project, it was agreed to use downy mildew of pansy (*Peronospora violae*), another host-specific downy mildew on an ornamental crop, as a model. As downy mildew pathogens are host specific, it was considered that the detection methods developed for pansy were likely to be applicable for use with a *Plasmopara* species on Impatiens, providing the PCR primers originally developed are capable of detecting a broad range of downy mildew species. The return of Impatiens downy mildew to the UK in 2007 and widespread infection in 2008 has meant that the project work has been divided and, to some extent, duplicated between both pathogen-host complexes.

There are 3 main aims to this project:

1. To develop a sensitive molecular method for detection of downy mildews, especially *Plasmopara obducens* and *P. violae* in seed/young plants.
2. To evaluate the safety and efficacy of a range of fungicides for effective disease prevention and control.
3. To develop an effective integrated GHP strategy which minimises the resistance risk and which can be quickly adopted by seed-houses, propagators/plug raisers, growers and commercial end-users of plants.

Summary of the project and main conclusions

Outbreak monitoring and isolate collection

A number of Impatiens plants heavily infected with *P. obducens* were submitted to the project team by commercial growers or their consultants via the Food and Environment Research Agency (Fera) & the STC diagnostic ‘Plant Clinic’. One batch

of infected Impatiens plants received exhibited an internal discolouration of the translucent stem tissues. This was subsequently shown to be the result of aggregations of resting or over-wintering survival spores (oospores) of the downy mildew pathogen *P. obducens*. This was the first record of *P. obducens* oospores on Impatiens grown in the UK. This finding may have significant repercussions in terms of pathogen persistence and subsequent spread due to the potential for longer-term survival of such oospores outdoors under UK conditions. The survival potential of the oospores of *P. obducens* has not been evaluated yet. Similarly, pansy plants infected by downy mildew were collected throughout the growing season, largely from growers, personal contacts and through local garden centres. Isolates were used in the development of plant infection protocols and in attempts to establish large scale infections in glasshouse trials.

Culturing/isolate maintenance

Leaf wetness was required for sporulation; high humidity alone was not sufficient. No sporulation occurred when leaves were incubated in the light or where stomata were covered by water, indicating that both initial sporulation and infection on pansy plants are likely to occur overnight Similar conditions were also required for the production of fresh spores of *P. obducens*. Freezing could not be used successfully as a method of long-term storage of either *P. violae* or *P. obducens* isolates. Isolates therefore have to be maintained through regular plant inoculation and transfer.

Plant infection

Infection of pansy plants by *P. violae* was observed following inoculation with freshly produced sporangia, provided that inoculated plants were kept at in the dark at 5°C for 20hrs to allow germination of sporangia. Downy mildew symptoms were first expressed after 10 days when plants were kept in the laboratory (mean daily temp 16°C) and 20 days when kept in the glasshouse (mean daily temp 8°C). Summing the mean daily temperature between inoculation and expression of symptoms for plants kept in the laboratory and plants kept in the glasshouse gave a thermal time of 170-degree days.

Infection of Impatiens by *P. obducens* was observed following inoculation with fresh sporangia provided inoculated plants were kept at in the dark at between 5 and 10°C for 20hrs to allow zoospore release and germination. Downy mildew symptoms were first expressed after 12 days, which related to a thermal time of 200-degree days.

Detection of Peronospora violae and Plasmopara obducens

Seed

Of the commercial Impatiens seed samples tested using molecular methods, 31% gave a result that indicated the presence of DNA of the downy mildew pathogen in/on the sample. This rate of DNA detection was lower than for the pansy seeds tested in the first year of the project, where 66% of the samples contained downy mildew DNA. To date, it has not been possible to produce downy mildew symptoms in either of the host plants grown from any of the seed lots containing downy mildew DNA. .

Seedlings

DNA analysis of pansy and Impatiens cotyledons and roots grown from seed lots with a range of downy mildew levels showed that, for both hosts, if downy mildew DNA was detected in a seed then it would also be detected in the seedling once grown-on. Interestingly, however, downy mildew DNA was detected more often, and at a higher frequency, in root material compared with the cotyledons. The significance of these findings is still not understood as the seedlings themselves did not express symptoms of downy mildew despite the presence of pathogen DNA.

Latent infection

The downy mildew primers and probes used in this project have proved capable of detecting latent infections of downy mildew in pansy leaf tissues. Levels of downy mildew DNA were shown to increase in the leaf material until the point when symptoms were expressed. This approach could therefore potentially be used in the future to 'screen' vegetative cutting material for the presence of the pathogen, though further validation would be required to develop it as a commercial service.

Fungicide trials

Crop Safety on Impatiens and pansy

Product safety was tested on young module-raised and semi-mature (in 6-pack) pansies and semi-mature (in 6-pack) Impatiens that were flowering at the point of application. All products were applied at standard (1xN) and twice standard (2xN) rate (see Table 1).

In general, all the products tested appeared to be relatively crop-safe on established plants. There was little visually obvious or consistent distortion or scorching of the foliage and flowers of either pansies or impatiens (although it should be noted that leaf scorching of pansy plants was consistently observed following the application of

Previcur Energy during the laboratory scale efficacy testing). However, significant reductions in plant height occurred in one pansy cultivar following some applications and chemical residue was present on the foliage following the application of Fubol Gold and Karamate Dryflo at the 2xN rate.

Growers considering using any of the products tested (noting that some of them are either experimental or can only be used on outdoor crops) should proceed with extreme caution and test-treat a few plants of different cultivars on their own nursery prior to widespread crop use.

It should also be noted that the Long Term Arrangements for Extension of Use (LTAEU) of pesticides on minor or specialist crops changed from 1st June 2009 and a number of products previously permitted via extrapolation from other edible crops can no longer be used. For a full list of the substances excluded from the LTAEU see http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/Appendix_1_Active_substances_excluded_from_the_LTAEU_from_1_June_2009.doc). Where possible, uses have been transferred to Specific Off Label Approvals (SOLAs). In some

Table 1. Fungicides and application used in pansy and Impatiens crop safety trials.

Product	Active ingredient	Application type	Application rate (mls or gms per litre)		Permitted 'commercial' use**	
			1xN	2xN	Protected crops	Outdoor crops
Subdue	metalaxyl-M	Drench	0.12 ml	0.24 ml	Yes (On-Label)	Yes (On-Label)
Amistar	azoxystrobin	Drench & spray	0.66 ml	1.3 ml	Yes (via SOLA 0443/09)	Yes (via SOLA 0443/09)
Bayer Exp.	imidazolinone gp. + ethyl-phosphonate	Drench & spray	3.0 g	6.0 g	No (experimental product)	No (experimental product)
Previcur Energy	propamocarb-HCL + fosetyl-al	Drench & spray	1.66 ml	3.32 ml	Yes (SOLA 2667/08)	Yes (SOLA 2667/08)
Epok	fluazinam + metalaxyl-M	Drench & spray	0.25 ml	0.5 ml	No	Yes (via extended LTAEU)
Revus	mandipropamid	Drench & spray	0.4 ml	0.8 ml	No	Yes (SOLA 2867/08 via LTAEU transfer)
Shinkon^	amisulbrom	Spray	0.33 ml	0.66 ml	No	Yes (via extended LTAEU)
Rose tonic*	potassium phosphate	Drench & spray	10 ml	20 ml	Yes (not a registered fungicide)	Yes (not a registered fungicide)
Fubol Gold	metalaxyl-M + mancozeb	Spray	1.25 g	2.5 g	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Olympus^	azoxystrobin + chlorothalonil	Spray	1.66 ml	3.32 ml	No	Yes (via extended LTAEU)
Stroby	kresoxim-methyl	Drench & spray	0.2 g	0.4 g	Yes (On-Label)	Yes (On-Label)
Shirlan#	fluazinam	Drench & spray	0.26 ml	0.52 ml	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Paraat	dimethomorph	Drench & spray	0.2 g	0.4 g	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Syngenta Exp.^	carboxylic acid amide+dithiocarbamate	Spray	1.66 g	3.32 g	No (experimental product)	No (experimental product)
Vitomex	phosphonic acid + derivatives	Spray	2.66 ml	5.32 ml	Yes (not a registered fungicide)	Yes (not a registered fungicide)
Karamate Dryflo[$]	mancozeb	Spray	1.8 g	3.6 g	Yes (On-Label)	Yes (On-Label)
Bravo 500[$]	chlorothalonil	Spray	2.2 ml	4.4 ml	Yes (On-Label)	Yes (but a different product[+])
Aliette 80 WG[$]	fosetyl-aluminium	Drench & spray	5 g	10 g	Yes (On-Label)	Yes (On-Label)
Filex[$]	propamocarb-HLC	Drench & spray	5 ml	10 ml	Yes (On-Label)	Yes (On-Label)
Serenade[$]	*Bacillus subtilis*	Spray	6.65 ml	13.3 ml	Yes (On-Label)	Yes (On-Label)
Croptex Fungex[$]	copper ammonium carbonate	Drench & spray	1.25 ml	2.5 ml	Yes (On-Label)	Yes (On-Label)
Thianosan DG[$]	thiram	Spray	4 g	8 g	Yes (On-Label)	Yes (On-Label)
Comet[$]	pyraclostrobin	Spray	0.66 g	1.3 g	No (experimental product)	No (experimental product)

[+] Only the product Juliet approved for use on outdoor ornamentals according to LIAISON.

* Only applied to semi mature Impatiens

[$] Only applied to young Impatiens

^ Not applied to young Impatiens

\# Potential for skin sensitisation in vulnerable individuals. This product should not be applied in situations where unprotected workers are handling plants.

** In many cases, subject to growers own risk.

cases, especially in protected ornamentals, this has not been possible due to concerns regarding operator or worker safety and growers or their advisers need to keep abreast of the latest situation to avoid application of non-approved products. The remaining active substances not included in this first round of evaluations will be processed over the next 5 years as the active ingredients come up for re-registration.

For further details of what Extension of Use arrangements remain after the 1st June 2009 see:-
http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/2nd_phase_product_list_27Jan2009_Web_version.xls.

Efficacy Testing against P.viola

Laboratory scale efficacy tests

Results from the laboratory-scale efficacy testing indicated that products containing metalaxyl-M (Fubol Gold and Subdue) were the most effective against pansy downy mildew, especially when applied as an eradicant spray (Fubol Gold) or as a soil drench (Subdue). However, there were a number of other products which also gave effective protectant control of the disease when applied at the manufacturer's recommended rate. These included Stroby (a.i. kresoxim-methyl), Olympus (a.i. azoxystrobin and chlorothalonil) and two experimental products (one produced by Bayer and the other by Syngenta). Products such as Previcur Energy (fosetyl-aluminium & propamocarb hydrochloride) and the non-pesticide products Rose Tonic and Vitomex and also gave good control of symptoms in laboratory-based studies. The wide range of active ingredients showing effective protectant control of the disease is encouraging as this means effective spray programmes, not relying on a single active ingredient, can be developed thus reducing the risk of resistant populations of downy mildew pathogens developing to metalaxyl-M, the most effective active ingredient.

Appropriate timing between applications for the different products

Products identified with potential for the control of downy mildew symptoms caused by *P. violae* were further examined to determine the appropriate time period between applications. Results indicated that products containing metalaxyl-M were the most effective, giving protection to a plant for a minimum of two weeks. The majority of the other products tested (Syngenta Exp, Stroby, Fubol Gold, Olympus, Rose Tonic and Bayer Exp) gave protection for a period of up to 7 days. These data suggest it would be possible to produce a 7 to 14 day spray programme capable of protecting a pansy crop from

infection by downy mildew, while minimising the risk of development of resistance to an individual fungicide active ingredient.

Financial benefits

The pathogens responsible for downy mildew are aggressive and, under favourable environmental conditions, they can cause significant economic losses. If we assume an industry value of £40M/annum for Impatiens alone (Coutts, pers comm.), and we estimate that some 10% plant losses of Impatiens may be incurred (this includes potential lost trade in subsequent years due to poorly performing plants in civic displays etc), then the financial benefit from a reduction in risk of disease in Impatiens alone could be as high as £4M/annum (in years where disease severity is high). Assuming losses also occur due to downy mildew infections in other crops e.g. pansy and viola, then the gross economic benefit of this R&D could be much higher.

Action points for growers

- Try to ensure any starting plant material (vegetative cuttings or seed) is disease free.
- Isolate and clearly label vegetative cutting and seed crops, including those from different suppliers to allow traceability should future problems arise.
- Ensure adequate air circulation around plants to minimise prolonged periods of leaf wetness by better spacing and by increasing the ventilation in the glasshouse. Avoid overhead watering as this is likely to aggravate the disease. If it is necessary to water from overhead systems then do this early, on days when solar radiation levels will ensure the leaves have a chance to dry out quickly.
- Be aware of criteria for sporulation and infection and check susceptible crops regularly, making arrangements for any suspicious plant material to be sent for diagnosis. Where infected plants are found remove them immediately by carefully placing them in a plastic bag *in situ* to avoid dispersing spores to other plants. Destroy any infected plants either by burial at landfill or via incineration.
- Maintain an effective fungicide programme on the crop, ensuring a range of products with different modes of action are included to minimise the risk of resistance development. Consider also the need for fungicides active against other important pathogens e.g. black root rot (*Thielaviopsis basicola*) & leaf-spot (*Ramularia* spp.) on pansy & viola.
- Prior to use of any fungicide for the first time test treat a few plants prior to widespread use on the crop to ensure freedom from phytotoxicity.

- Practice good nursery hygiene, clean up crop debris between crops and at the end of the season to minimise the risk of carry-over of the disease and maintain effective weed control (including ‘volunteer’ Impatiens plants) in and around the growing areas. Use appropriate disinfectants responsibly to help minimise potential carry-over of inoculum.
- Where infected material is found notify the project team and, if possible, submit a sample for R&D purposes.
- Keep abreast of developments with changes to the Long Term Arrangements for Extension of Use (LTAEU) arrangements.

Science Section

1. Introduction

Downy mildew on *Impatiens walleriana*, was first reported in the UK by STC (McPherson & Finlay, pers. comm.) in June 2003 causing considerable economic damage to commercial crops and municipal plantings, especially, though not exclusively, in the South of England. STC provided a presumptive identification as *Plasmopara obducens* and, as it was considered unusual and a potential first UK record, Plant Health were notified, The identity of the fungus was subsequently confirmed as *P. obducens* by CSL (Lane *et al.*, 2005). Emergency statutory action was immediately taken by the Plant Health & Seeds Inspectorate (PHSI) under Article 22 of the Plant Health Order 1993 and the downy mildew pathogen on impatiens was declared notifiable. This emergency legislation required the industry to notify PHSI of any suspect cases of the disease and, where the pathogen was confirmed, statutory action was taken to destroy the infected plants and to quarantine (for a pre-determined time period) the remaining apparently disease-free stock. If the pathogen was subsequently found to have spread to adjacent stock this would also be destroyed. The cost of such crop loss had to be met by the individual grower, as government compensation for crop loss was not available. A cost-benefit analysis of management options for the control of impatiens downy mildew was undertaken (Jones, 2004) which concluded that: "the most cost-effective option for government would be for the industry to police itself in the future as regards this disease". This was a view supported by the industry and in May 2005, Defra announced that statutory action would no longer be taken and that the industry would take on responsibility for management of the disease through implementation of an industry code of practice (Good Horticultural Practice (GHP), McPherson and Brough, 2009).

According to the scientific literature, *P. obducens* is reported to occur in North America and parts of Asia and Europe, including Denmark, Finland, the Netherlands, Germany, the Czech Republic and Lithuania in the EU and Romania and Russia in the rest of the EPPO region. In reality, the disease is much more widespread than this and in 2008, also caused problems in South Africa, Australia and Japan. The full extent of its distribution in the UK is not clear as it may also occur on natural or introduced relatives of Impatiens found in the wild e.g. *Impatiens noli-tangere and I. glandulifera*; however, in limited observations to date no such findings have been made. Commercial trade of impatiens, valued at around £40M/annum, is complex and involves seed houses, specialist propagators (often overseas), growers, the retail trade (including garden centres & mail order enterprises) and municipal production for civic displays in parks etc. (S. Coutts, pers. comm.). This chain of

production is highly relevant and important with respect to the potential for pathogen introduction, subsequent disease spread and the future successful implementation of various control measures.

In the original UK outbreaks in 2003, it is suspected that *Impatiens* raised from seed or, more likely, as imported vegetative transplants (unrooted or rooted cuttings) may have provided the initial infection source of the disease, though this has not been confirmed. Over-wintering or resting spores (oospores) of the pathogen have previously been reported on/in seed in India, but their absence in early UK outbreaks indicated perhaps that the pathogens potential to survive over-winter under UK conditions was low. However, in 2008 the situation in the UK changed considerably when, for the first time, resting spores were found in infected stem tissues of *I. walleriana* (Turner *et al*, 2009). Given this new finding, the potential risk of carry-over between seasons on plant debris incorporated into the soil has increased significantly. The potential for seed-borne transmission via this route is also potentially increased, though it must be emphasised that, as yet, oospores have not been found associated with seed-lots of *I. walleriana* or other species and cultivars in the UK.

In situations where resting spores (oospores) maybe incorporated into the soil with infected plant debris there is a potential increased risk of direct infection to subsequent outdoor planting schemes. However, how big this risk is, relative to directly sown seed crops, remains unknown. It is considered that the risk of systemic infection of established plants from 6-packs by germinating oospores in the soil is relatively low, but repeat planting in situations where the disease was present the previous season should be avoided where possible.

Infected seed is reported to give rise to systemically infected plants and a long latent period between infection and appearance of symptoms has also been observed. This could potentially lead to long-distance dissemination of the pathogen in consignments of seed and plants that appear disease-free on delivery.

When this project was commissioned, occurrences of impatiens downy mildew throughout Europe remained low, which made it very difficult to progress the project due to the lack of availability of isolates and infected host material from diseased plants. The pathogen involved is an obligate organism and therefore cannot be retained in artificial culture. In order to ensure progress could be maintained within the project, it was therefore agreed to use another host-specific downy mildew on an ornamental crop as a model. Downy mildew

of pansy (*Peronospora violae*) was identified as an alternative test system as this disease is endemic within UK grown pansies and continues to cause considerable economic losses in some seasons and on some nurseries requiring prophylactic fungicide application for control. As downy mildews are host specific, the detection methods developed for pansy would also be applicable for use with a *Plasmopara* species on impatiens, providing the PCR primers originally developed were capable of detecting a broad range of downy mildew species. New findings of impatiens downy mildew in the UK in 2007 and , and on a much larger scale in 2008, has resulted in the experimental work being split between the two diseases.

In order for the industry to take responsibility for the overall management of downy mildew on impatiens and pansies, it is necessary for growers to have at their disposal effective techniques for the detection of early (latent) infections of downy mildew on both seed and young propagation material and also access to a range of approved crop protection products with demonstrated activity and crop safety against *Plasmopara obducens* and *Peronospora violae*. Any recommended spray programme must also consider the resistance risk to some fungicide groups e.g. phenylamides and ensure a range of products with different modes of action are available to minimise the selection of tolerant and/or resistant strains in the pathogen population.

The primary aims of the proposed project were to investigate techniques for the early detection and control of downy mildew in order to provide the industry with appropriate tools for effective disease management. The objectives were to develop a sensitive molecular method for detection of the downy mildew fungus in seed/young plants and to evaluate the efficacy and safety of a range of fungicides for effective control of the disease culminating in the development of an integrated strategy to minimise resistance risk.

2. Materials and Methods

Culturing and maintenance

Plant infection studies - Impatiens.

Impatiens plants (c.v. DeZire various colours) with downy mildew symptoms were sent to Fera on the 10th August 2009. Symptoms on these plants, unlike those sent to the Laboratory in 2007 and 2008, appeared to be recently developed, with sporulation occurring only in small discrete areas on the underside of some of the leaves.

All sporulating leaves were removed from the plant and a sub-set of these incubated at 18°C in moist chambers for between 20 and 24 hrs in the dark to stimulate production of fresh spores. Spores were washed separately with sterile distilled water (SDW) from both the incubated leaves and those taken directly from the plant. Two sets of two impatiens plants were inoculated to run off, one with the fresh spores and the other with the 'older' spores. Both sets of inoculated plants were divided into two, and one set of each incubated in the dark at 5°C for 20 h and the other in the dark at about 14°C (overnight ambient temperature); plants were incubated in propagator tops to prevent drying out of the inoculum. All plants were then transferred to a glass roofed gauze house and monitored for development of symptoms.

The remaining sporangial suspension from the plant inoculation was incubated under the same initial conditions as the inoculated plants (in the dark for 20 hours at 5 or 14°C). Sporangia were then examined under the microscope to determine whether spore germination had occurred.

Sporangial germination - Impatiens

Following the removal of sporulating leaves for the inoculation studies the impatiens plants were stored in the dark at 5°C. Examination of plants 48 hours later revealed sporulation on leaves which had not previously shown any signs of infection (yellowing of leaves or sporulation). Sporangia were washed from the leaves in SDW divided into three equal volumes and incubated at 5, 10 or 18°C for 2 h, after which the sporangia were examined under the microscope for signs of germination.

Isolate storage

Plasmopara obducens

In previous years, it has been reported that the freezing of sporulating leaf material could not be used as a method for the maintenance of *P. obducens* isolates. However, evidence

from the final year of the project suggests that the inoculum previously frozen may not have been viable prior to storage. In light of this, impatiens leaf material with freshly produced sporangia, known to be viable, were frozen to once again try and determine the suitability of the method for longer-term storage of isolates. After 10 days at -25°C impatiens leaf material was removed from the freezer and allowed to thaw, sporangia were washed from the leaf and incubated at 10°C for 2h. Sporangia were then examined under the microscope to examine viability.

Peronospora violae

As with *P. obducens*, freezing had not proven to be an effective method for the storage of *P. violae* isolates - possibly due to the lack of spore viability. As methods have now been developed for the routine production of viable sporangia of *P. violae,* the potential for freezing as a storage method was tested again. Methods used were similar to those described in section 2.1.3, except that sporangial viability was measured following incubation at 5°C for 20h.

Effect of fungicides against the pansy downy mildew pathogen *Peronospora violae*.

Fungicide efficacy testing - glasshouse (small scale) (Fera)

A total of 14 fungicides (and related products) (13 as foliar sprays and five as drench applications) were tested for efficacy against the Pansy downy mildew pathogen *Peronospora violae* (Table 1). All products were applied according to the manufacturer's recommended rate). Foliar sprays were applied to run off and soil drenches were applied at volumes corresponding to 10% of the pot volume (with the exception of Bayer Exp which was applied at 100 mL L^{-1} of compost). Both foliar and drench treatments were applied at two timings, three days pre or three days post inoculation. Control plants were either sprayed or drenched with an equivalent volume of SDW.

Fresh inoculum was produced and plants inoculated as described above. For each fungicide and control treatment, three replicate pansy plants were inoculated with the sporangial suspension to run-off, ensuring that both the abaxial and adaxial surfaces of the leaf were inoculated. Plants were transferred to a propagator top and incubated at 5°C in the dark for 20h and before being placed in a glass-roofed gauze house. Plants were assessed for downy mildew symptoms after approximately 170 degree days (time at which the sum of the mean daily temperature reached 170) and again one week later. At each assessment, five random leaves per plant were examined for the presence of downy mildew symptoms.

Table 1. Fungicides used in efficacy testing against *Peronospora violae*.

Product	Active ingredient	Application type	Application rate (L^{-1})
Subdue	metalaxyl-M (480 g L^{-1})	Drench	0.12 mL
Fubol Gold	metalaxyl-M (40 g kg^{-1}) + mancozeb (640 g kg^{-1})	Foliar spray	1.25 g
Amistar	azoxystrobin (250 g L^{-1})	Foliar spray	0.66 mL
Bayer Exp.	imidazolinone group + ethyl-phosphonate	Foliar spray Drench	3.0 g 1.5 g
Previcur Energy	propamocarb-HCL + Fosetyl-al	Foliar spray Drench	1.66 mL 1.66 mL
Revus	mandipropamid	Foliar spray	0.4 mL
Shinkon	amisulbrom	Foliar spray	0.33 mL
Rose tonic*	potassium phosphate	Foliar spray Drench	10 mL 10 mL
Olympus	azoxystrobin + chlorothalonil	Foliar spray	1.66 mL
Stroby	kresoxim-methyl	Foliar spray	0.2 g
Shirlan	fluazinam	Foliar spray	0.26 mL
Paraat	dimethomorph	Foliar spray Drench	0.2 g 0.2 g
Syngenta Exp.	carboxylic acid amide + dithiocarbamate	Foliar spray	1.66 g
Vitomex	phosphonic acid + derivatives	Foliar spray	2.66 mL

Effect of application timing on fungicide efficacy – glasshouse (small scale) (Fera)

Following the initial efficacy trials, six foliar spray (Syngenta Exp, Stroby, Fubol Gold, Olympus, Rose Tonic and Bayer Exp) and two drench (Subdue and Previcur Energy) applications were selected to test pre-inoculation timings, and five spray (Fubol Gold, Previcur Energy, Rose Tonic and Vitomex) and two drench (Subdue and Bayer Exp) applications were chosen to evaluate post-inoculation timings. Timings used were 3, 7 and 14 days pre inoculation, and 3, 7 and 10 days post inoculation; the 14 day post application timing was not used to ensure downy mildew symptoms were not expressing prior to treatment. Three replicate pansy plants were used for each control, fungicide treatment and application timing.

Inoculum production, inoculation, fungicide application and assessment methodologies were as described in section 2.3.1.

Efficacy study – Glasshouse (large scale) (STC)

A total of 16 spray programmes (9 using a single product and 7 using a combination of products, Table 2) were tested for effectiveness in protecting a pansy crop from downy mildew infections caused by *P. violae* using a 4 spray programme applied at 7-14 day

intervals. The spray treatments were applied with a Hozelock hand sprayer to produce a fine spray and were applied to run-off (0.5L/16 six packs). Application intervals were determined by the level of downy mildew infection developing in the crop, with a minimum of 7 days between sprays. Control plants were sprayed with water at each of the fungicide timings. Each programme was replicated four times with each programme plot consisting of four six packs of pansies (two planted with Skyline True Blue and two with Rocky Deep Blue Blotch). A larger number of plots than required were laid out to ensure an entire glasshouse bay was filled (this helped increase humidity and aided the development of downy mildew infection); the extra plots were interspersed at random within the trial (see trial plan Appendix 1). The trial was laid out in a cool, shaded glasshouse on a soil floor (Figure 1); this ensured cooling of the house and also the use of overhead irrigation in adjoining bays to raise humidity, again to aid disease development.

The trial area was inoculated 2 days after laying out of plants in the glasshouse, using a spore suspension produced from infected pansy plants at Fera. The first fungicide spray was applied 6 days later. The crop was monitored daily for the development of downy mildew infection. Throughout the growing period, flowers were removed from the trial plants in an effort to keep them vegetative and more susceptible to infection. The trial plants were also trimmed back on the 11th August to encourage new soft growth which would be more likely to become infected with downy mildew. The plants in the spare plots were left untrimmed to ensure that there was adequate leaf material to be infected during the scheduled second inoculation (see crop diary below).

Crop Diary

8.5.09	1500 Skyline True Blue and 1500 Rocky Deep Blue Blotch pansies sown
17.6.09	Pansy plugs potted into 6 packs
30.6.09	Plants moved to MFU glasshouse and trial laid out.
7.7.09	1st inoculated with DM spore suspension
13.7.09	1st fungicide spray application
21.7.09	Application of PGR to crop (3g/L)
11.8.09	Crop trimmed to produce new growth
21.8.09	2nd inoculation with DM spore suspension.
8.9.09	Trial terminated.

Table 2. Spray programme intended for use during glasshouse scale efficacy study.

Programme	Product used in spray programme	Active Ingredient	Application Rate/L
T1	Untreated	water	-
T2	Serenade x 4	*B. subtilis*	6.6 ml
T3	Karamate x 4	mancozeb	1.8 g
T4	Amistar x 4	azoxystrobin	0.66 ml
T5	Stroby x 4	kresoxim-methyl	0.2 g
T6	Signum x 4	pyraclostrobin + boscalid	0.9 g
T7	Revus x 4	mandipropamid	0.5 ml
T8	Epok x 4	fluazinam +metalaxyl-M	0.25 ml
T9	Paraat x 4	dimethomorph	0.2 g
T10	a) Previcur Energy[#]	propamocarb-HCl + fosetyl-al	1.66 ml
	b) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
	c) Karamate	mancozeb	1.8 g
	d) Amistar	azoxystrobin	0.66 ml
T11	a) Subdue*	metalaxyl-M	0.125 ml
	b) Karamate	mancozeb	1.8 g
	c) Karamate	mancozeb	1.8 g
	d) Previcur Energy	propamocarb-HCl + fosetyl-al	1.66 ml
T12	a) Amistar	azoxystrobin	0.66 ml
	b) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
	c) Amistar	azoxystrobin	0.66 ml
	d) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
T13	a) Stroby	kresoxim-methyl	0.2 g
	b) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
	c) Stroby	kresoxim-methyl	0.2 g
	d) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
T14	a) Signum	pyraclostrobin + boscalid	0.9 g
	b) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
	c) Signum	pyraclostrobin + boscalid	0.9 g
	d) Fubol Gold	metalaxyl-M + mancozeb	1.25 g
T15	a) Serenade	*B. subtilis*	6.6 ml
	b) Amistar	azoxystrobin	0.66 ml
	c) Serenade	*B. subtilis*	6.6 ml
	d) Subdue	metalaxyl-M	0.125 ml
T16	a) Serenade	*B. subtilis*	6.6 ml
	b) Revus	mandipropamid	0.5 ml
	c) Serenade	*B. subtilis*	6.6 ml
	d) Paraat	dimethomorph	0.2 g

* Subdue is normally applied as a drench, but is applied also as a HVS in various treatments experimentally.

applied as drenches at 10% module soil volume

Figure 1. General shot of pansy efficacy trial carried out at STC.

Crop safety trial – STC

A final crop safety trial was carried out on plug plants of *Impatiens walleriana* and a range of *I. hawkeri* New Guinea types. The trial on young plug plants was carried out to provide crop safety data on effects on young foliage, flowers and plants in bud. Trays of DeZire (Need consistency in the report is it Dezire or DeZire?) Select Mix and of 9 colours of the Harmony Series of New Guinea impatiens were used. The colours included in the investigation were Dark Lavender, Magenta, Dark Red, Purple Eye, Pink Smile, Boogie, Scarlet, Cherry Ice and Orange Blaze. Each plot consisted of 1/3rd of a tray of *I. walleriana* (120 plugs) and 1 plug of each of the New Guinea types. The trial was laid out in a fully randomised block design and maintained in an ambient glasshouse with venting for the duration of the trial (Figure 2).

Nineteen products were included in the crop safety studies (Table 3). Metalaxyl-M (as Subdue) and metalaxyl-M + mancozeb (as Fubol Gold) were included as standard drench and spray treatments respectively and were used alongside the water control for comparison purposes. The remainder of the products were included either for their known control of downy mildew pathogens or control of other oomycete pathogens e.g. *Phytophthora infestans* in potato, and included one experimental (Bayer) and two biological or 'alternative' products (Serenade and Vitomex). Products were applied either as a drench or foliar spray depending on their label recommendation or potential use. Drenches were applied at 10% of the module compost volume whilst spray treatments

used a water rate of approx 1500 L/ha using hand pumped Hozelock sprayer. Products were applied at the normal (1 x N) rate and 2 x N rate. Following fungicide application, plants were monitored for visual signs of phytotoxicity e.g. stunting, scorch, leaf or flower distortion.

Figure 2. General shot of crop safety trial carried out at STC on young impatiens.

Table 3. Fungicides and application used in crop safety trials on young impatiens

Product	Active ingredient	Application type	Application rate (per litre)	
			1xN	2xN
Subdue	metalaxyl-M	Drench	0.12 ml	0.24 ml
Amistar	azoxystrobin	Drench & spray	0.66 ml	1.3 ml
Bayer Exp.	imidazolinone gp. + ethyl-phosphonate	Drench & spray	3.0 g	6.0 g
Previcur Energy	propamocarb-HCL + fosetyl-al	Drench & spray	1.66 ml	3.32 ml
Epok	fluazinam + metalaxyl-M	Drench & spray	0.25 ml	0.5 ml
Revus	mandipropamid	Drench & spray	0.4 ml	0.8 ml
Fubol Gold	metalaxyl-M + mancozeb	Spray	1.25 g	2.5 g
Stroby	kresoxim-methyl	Drench & spray	0.2 g	0.4 g
Shirlan#	fluazinam	Drench & spray	0.26 ml	0.52 ml
Paraat	dimethomorph	Drench & spray	0.2 g	0.4 g
Vitomex	phosphonic acid + derivatives	Spray	2.66 ml	5.32 ml
Karmate Dryflo	mancozeb	Spray	1.8 g	3.6 g
Bravo 500	chlorothalonil	Spray	2.2 ml	4.4 ml
Aliette 80 WG	fosetyl-aluminium	Drench & spray	5 g	10 g
Filex	probamocarb-HLC	Drench & spray	5 ml	10 ml
Serenade	*Bacillus subtilis*	Spray	6.65 ml	13.3 ml
Croptex Fungex	copper ammonium carbonate	Drench & spray	1.25 ml	2.5 ml
Thianosan DG	thiram	Spray	4 g	8 g
Comet	pyraclostrobin	Spray	0.66 g	1.3 g

3. Results and Discussion

Culturing and maintenance

Sporangial germination - Impatiens

Zoospore release was observed from sporangia incubated at 5 and 10°C but not 18°C (Figures 3-5); zoospore release was earliest from sporangia incubated at 10°C. Zoospores were not released from sporangia incubated at 18°C, even after 48 h. The mycelial germination of sporangia found with *P. violae* was not observed for *P. obducens*. Transfer of sporangia previously kept in water at 18°C for 48h to 10°C resulted in zoospore release after 1.5h. On average each sporangium released four zoospores, which became spherical (approx. 9 μm diameter) shortly before encysting.

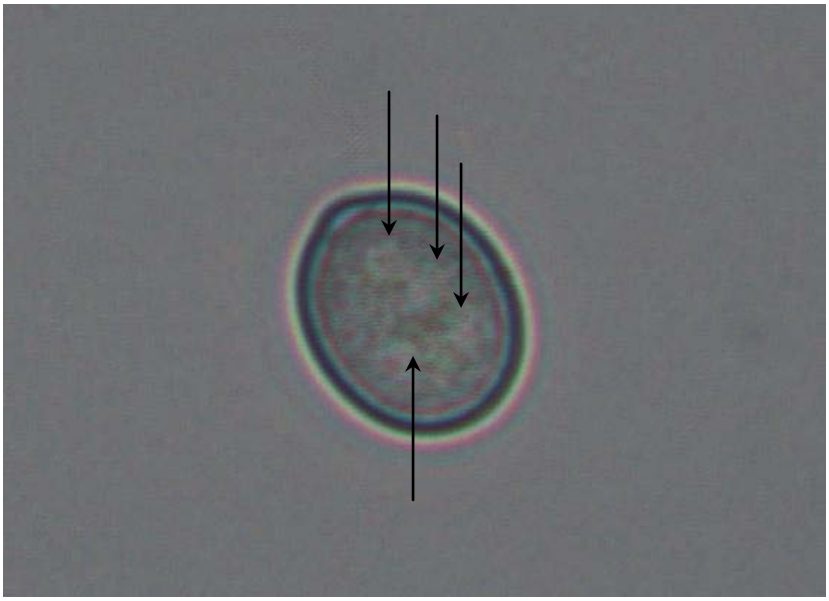


Figure 3. *Plasmopara obducens* sporangium containing four zoospores (indicated by arrows).

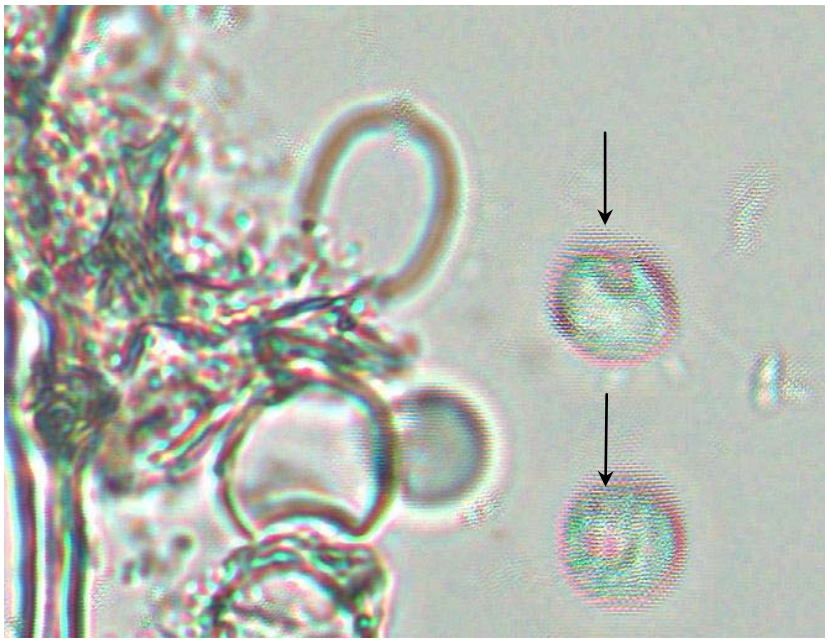


Figure 4. *Plasmopara obducens* sporangiophore (far left), empty sporangia (centre) and free-swimming zoospores (arrowed).

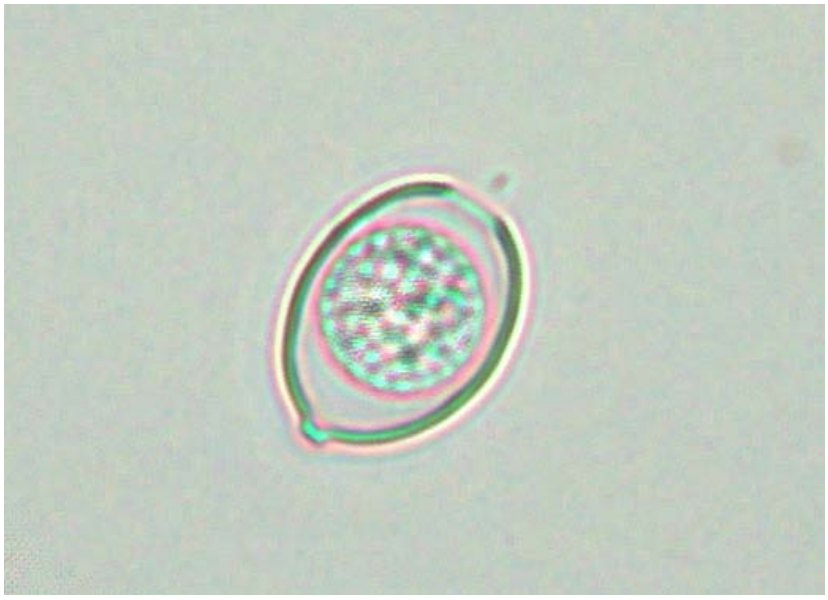


Figure 5. *Plasmopara obducens* sporangium with a single encysted zoospore remaining inside.

No sporangial germination was detected in any potential inoculum from impatiens downy mildew infections sent to the laboratory in years 1 and 2 of the project. The difference between the samples obtained in the first two years and the one obtained in the final year of the project appears to be the age of the infection. In the first two years, the plants received had infections, which appeared advanced, with sporulation occurring over the entire underside of the leaves causing widespread leaf drop, whereas symptoms on the

plant obtained in the third year appeared to be still developing as only discrete areas of sporulation were found on the underside of leaves. These observations seem to suggest that sporangia produced by *P. obducens* are short lived and that if conditions suitable for their spread are not available they soon lose viability.

Infection and germination studies undertaken here indicate a temperature range between 5 and 14°C over which zoospores of *P. obducens* are released from detached sporangia, with the optimum temperature for release around 10°C. Generally, these temperatures are similar to those seen for another *Plasmopara* species, *Plasmopara viticola* the causal agent of downy mildew on grape vines, where sporangial differentiation and zoospore release occurred between 10 and 16°C in distilled water (Santilli, 1958).

Plant infection studies -Impatiens

Symptoms of impatiens downy mildew (Figure 6) were recorded on all inoculated plants 12 days after inoculation; this was equivalent to a thermal time 200-degree days. The level of downy mildew symptoms observed was greater on plants held at 5°C for 20h following inoculation compared to those held at 14°C. No obvious symptoms were noted on plants prior to development of sporulation on the underside of the leaves.

Figure 6. Downy mildew symptoms on underside of an impatiens leaf inoculated with sporangia of *Plasmopara obducens*.

Microscopic examination of older and freshly produced sporangia incubated under the same initial conditions as the inoculated impatiens (5 and 14°C in the dark for 20 h) showed that the vast majority of sporangia were empty (Figure 7) suggesting that zoospores had been released. This was the first observation of zoospore release from

sporangia of *P. obducens* in the project and suggested the inoculum used for plant inoculation was viable and that the conditions used were likely to be suitable for infection of the inoculated plants.

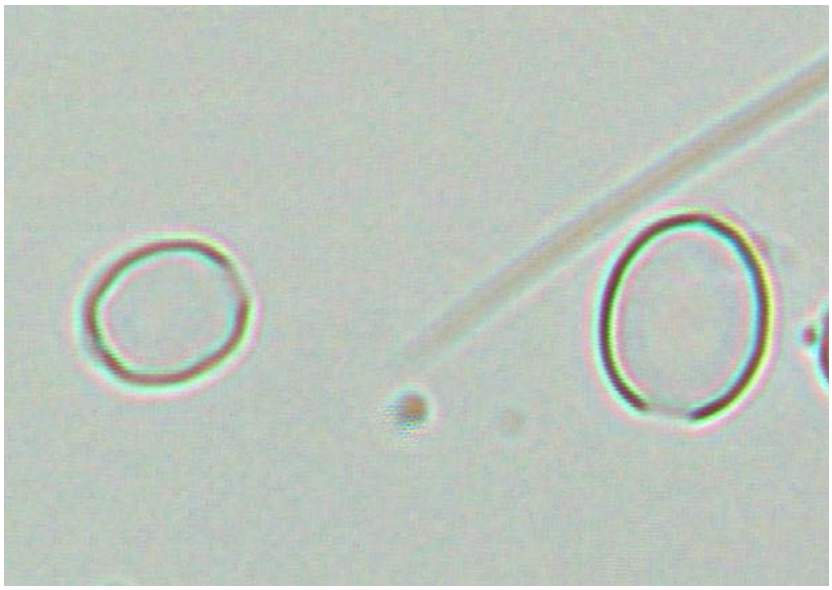


Figure 7. Empty sporangia of *Plasmopara obducens* following incubation in the dark at 5°C for 20 h.

Isolate storage

Plasmopara obducens

Zoospore release was not observed following the thawing of young *P. obducens* sporangia previously frozen for 10 days at -25°C, indicating that the sporangia were no longer viable. This further indicates that freezing of sporulating leaf material is not an option for the long-term storage of *P. obducens* and that preservation of isolates will have to be through maintenance on plants.

Peronospora violae

No germination was observed following the thawing of young *P. violae* sporangia previously frozen at -25°C for 10 days, indicating that the sporangia were no longer viable. As for *P. obducens*, this again indicates that freezing of sporulating leaf material is not an option for the long-term storage of *P. violae* and that preservation of *P. violae* isolates will have to be through maintenance on plants.

Effect of fungicides against the pansy downy mildew pathogen *Peronospora violae*.

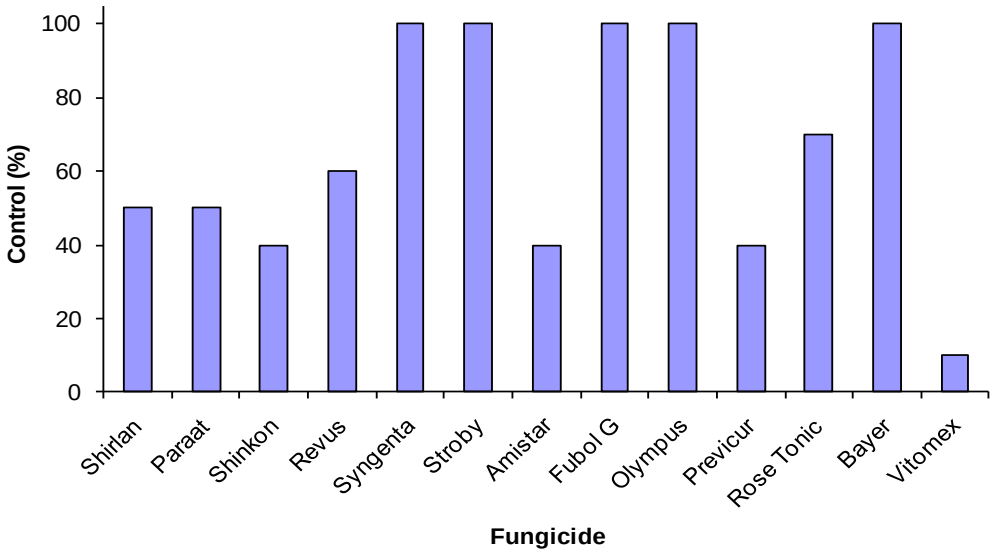
Even though criteria for producing downy mildew infections on impatiens were established in the final year of the project it was too late to for any fungicide efficacy trials to be carried

out on impatiens. As a result, such trials were only carried out on downy mildew infections of pansies.

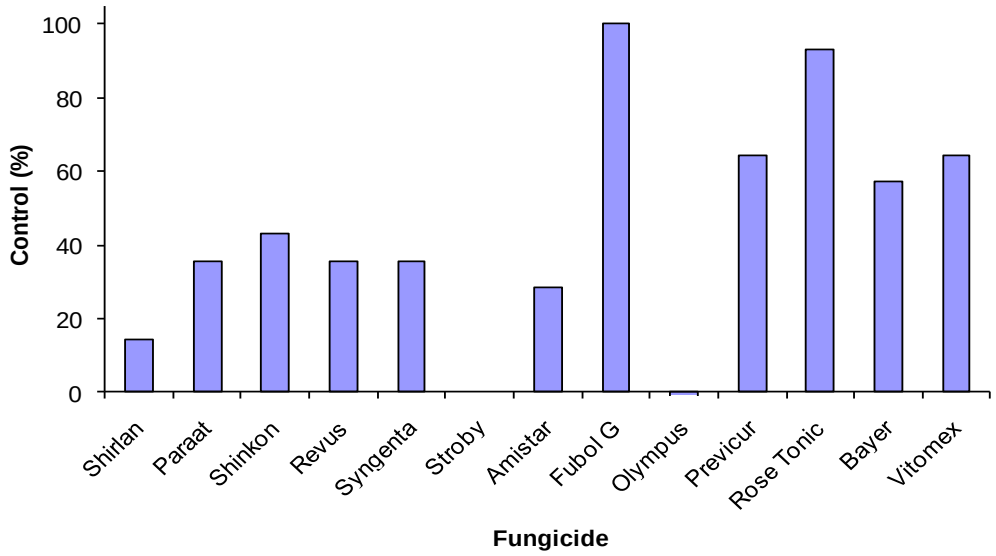
Fungicide efficacy testing – glasshouse (small scale) (Fera)

Thirteen foliar spray products were tested for efficacy against *P. violae* when applied either 3 days pre or 3 days post inoculation. The products tested showed a wide range of activity, with all products reducing symptoms compared to controls when applied to foliage prior to inoculation (Figure 8a). Products were generally more active when applied as a protectant (pre-inoculation) than as a curative (post-inoculation) treatment (Figures 8a and b). Five products (Syngenta Exp (carboxylic acid amide & dithiocarbamate), Stroby (kresoxim-methyl), Fubol Gold (metalaxyl-M), Olympus (azoxystrobin & chlorothalonil) and Bayer Exp (imidazolinone grp & ethyl-phosphonate)) gave complete control of pansy downy mildew symptoms when applied as a foliar spray 3 days pre inoculation (Figure 8a). Of these products only Fubol Gold (containing metalaxyl-M) gave the same level of control when applied to the foliage post inoculation (Figure 8b), whereas the remaining four products all gave less than 60% control, with Stroby and Olympus giving no control of symptoms compared to the control plants. Three products (Previcur Energy, Rose Tonic and Vitomex) performed better when applied to foliage as a post inoculation treatment, and a single product, Shinkon (amisulbrom), gave similar levels of control at both timings.

Five products were tested as drenches for efficacy against *P. violae*. Of the products tested, Subdue (metalaxyl-M) was the most effective treatment with no development of downy mildew symptoms after treatment at either timing (Figure 9a and b). Of the remaining products, Previcur Energy gave the greatest control when applied pre-inoculation, whereas Bayer Exp and Rose Tonic worked best when applied post-inoculation. Paraat (dimethomorph) was ineffective as a soil drench and did not reduce downy mildew symptoms compared to the control plants.



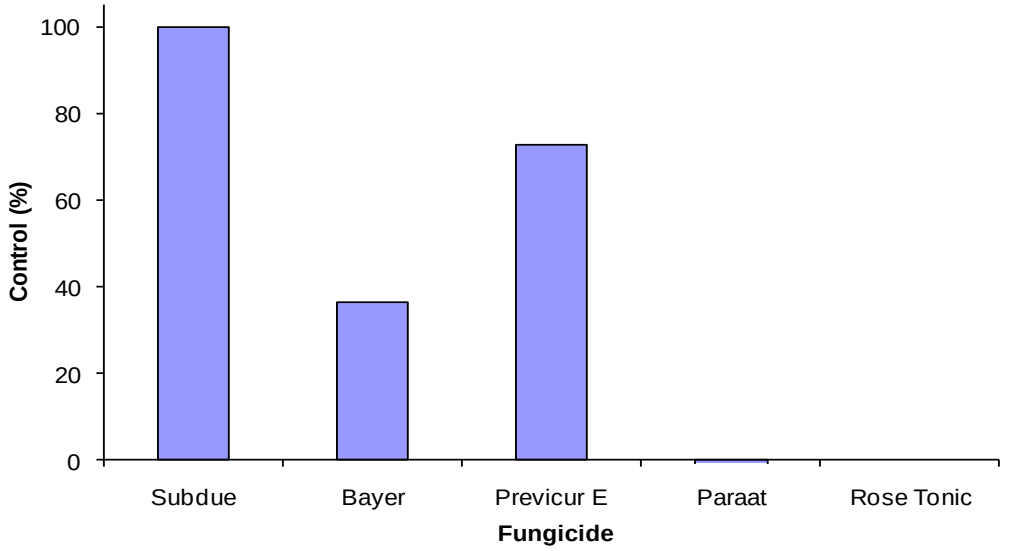
a)



b)

Figure 8. Efficacy of foliar fungicide applications a) 3-days pre and b) 3-days post inoculation of pansy plants with sporangia of *Peronospora violae*.

a)



b)

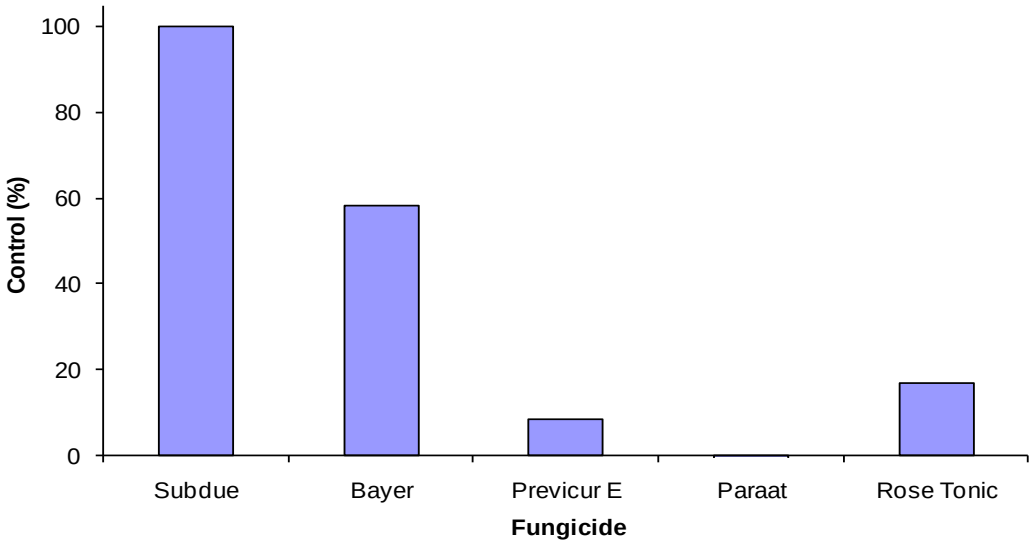


Figure 9. Efficacy of soil drench fungicide applications a) 3-days pre and b) 3-days post inoculation of pansy plants with *Peronospora violae* sporangia

During the course of these tests some phytotoxicity effects became apparent that had not been observed during trials carried out in the second year of the project. These were observed particularly following the application of Previcur Energy as a foliar spray when scorching of the leaf margins was commonly noted (Figure 10).

Figure 10. Leaf margin scorching (indicated by arrows) observed following the application of Previcur Energy as a foliar spray

Results from the laboratory-scale efficacy testing indicated that products containing metalaxyl-M (Fubol Gold and Subdue) were the most effective against pansy downy mildew, especially when applied as an eradicant spray (Fubol Gold) or as a soil drench (Subdue). However, there were a number of other products, which also gave effective protectant control of the disease when applied either at the label rate or the rate proposed by the manufacturer (i.e. experimental products). These included Stroby, Olympus and two experimental products (one produced by Bayer and the other by Syngenta). Products such as Rose Tonic, Vitomex and Previcur Energy also gave good control of symptoms. The wide range of active ingredients showing effective protectant control of the disease is encouraging as this means effective spray programmes, not relying on a single active ingredient, can be produced thus reducing the risk of resistant populations developing to metalaxyl-M, the most effective active ingredient.

Effect of application timing on fungicide efficacy – glasshouse (small scale) (Fera)

Products identified with potential for the control of downy mildew symptoms caused by *P. violae* were further examined to determine the appropriate time period between applications. Timings were evaluated for six foliar spray (Syngenta Exp, Stroby, Fubol

Gold, Olympus, Rose Tonic and Bayer Exp) and two drenches (Subdue and Previcur Energy) as protectant treatments, and five foliar spray (Fubol Gold, Previcur Energy, Rose Tonic and Vitomex) and two drenches (Subdue and Bayer Exp) as eradicant treatments.

Fubol Gold was the most effective of the protectant foliar sprays providing 100% control of downy mildew symptoms even when applied 14 days before inoculation (Figure 11). For all other products the level of control reduced as the time between application and inoculation increased. Foliar applications of Syngenta Exp, Bayer Exp, Olympus and Rose Tonic all gave 100% control of symptoms up to 7 days pre-inoculation, however the control achieved varied between 20 and 60% when the timing was stretched to 14 days. Stroby was the least effective of the pre-inoculation foliar sprays, giving 100% control at the 3 day timing, however stretching the time between inoculation and application to 7 or 14 days resulted in 80 and 0% control respectively.

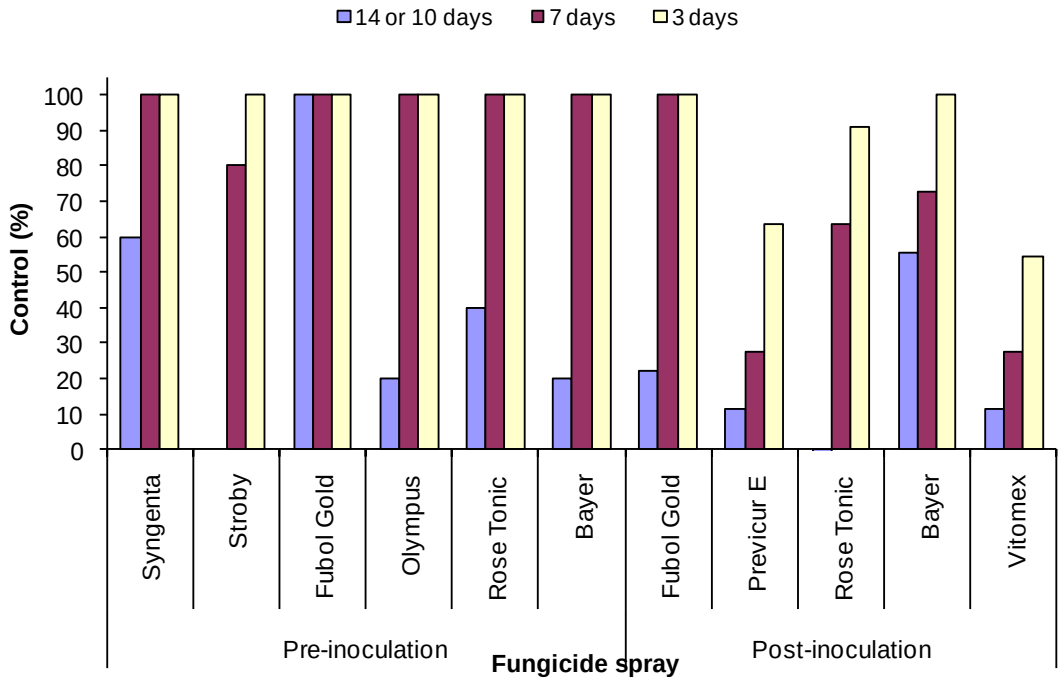


Figure 11. The effect of pre- and post-inoculation foliar application timings on control of pansy downy mildew symptoms caused by *Peronospora violae*.

The first sign of symptoms were first observed in control plants 11 days after inoculation. As with the protectant treatments the level of disease control achieved with eradicant treatments reduced as the time period between inoculation and product application increased (Figure 11). As seen in the initial efficacy trial, the control achieved following an

eradicant treatments was lower than following a protectant treatment. Fubol Gold was the most effective of the eradicant sprays giving 100% control at 3 and 7 days, however control was reduced to 20% using the 10 day eradicant spray (Figure 11). No other product gave 100% control when applied after 7 days and only Bayer experimental product gave 100% control at the 3 day timing.

The only soil drench application to give 100% control of pansy downy mildew symptoms was Subdue (ai metalaxyl-M). The only application timing when this product did not result in 100% control was when the treatment was delayed until 10 days after inoculation (Figure 12).

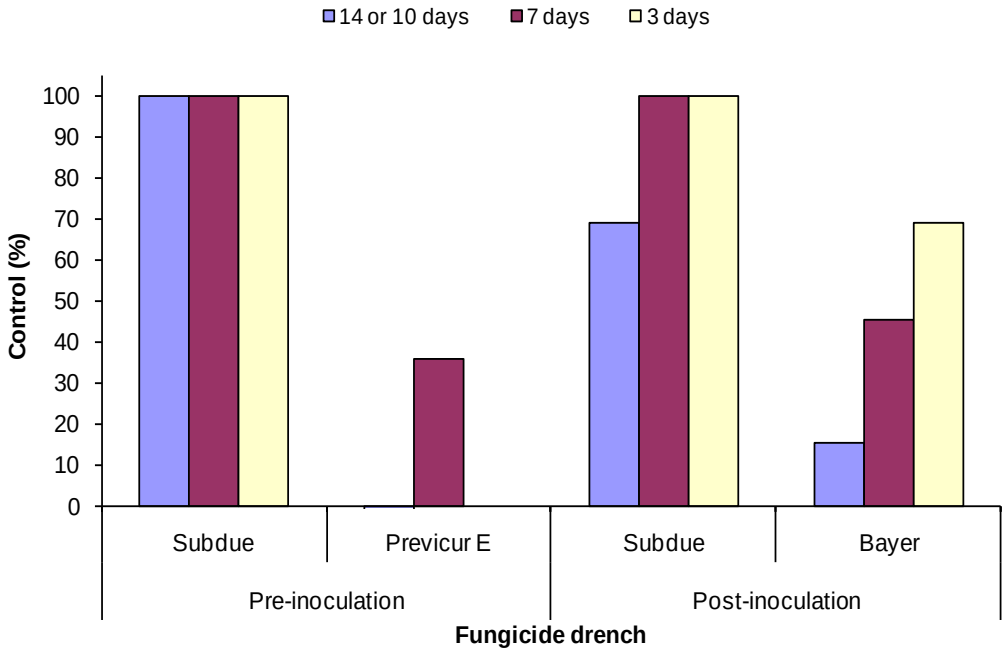


Figure 12. The effect of pre- and post-inoculation soil drench application timings on control of pansy downy mildew symptoms caused by *Peronospora violae*.

Results from the application timing trial indicate that products containing metalaxyl-M were the most effective, giving protection to a plant for a minimum of two weeks. It is likely that other metalaxyl-M containing products e.g. Epok (fluazinam/metalaxyl-M) will also be as effective against downy mildew. This is especially true where the product formulation contains a higher level of metalaxyl-M, e.g. Epok contains 200 g L^{-1} metalaxyl-M compared to the tested product (Fubol Gold), which contains 40 g L^{-1}. The majority of the remaining products tested gave protection for a period of up to 7 days. These data suggest that several foliar spray fungicides are capable of protecting a pansy crop from downy mildew

infections so it would be possible to produce a 7 to 14 day spray programme, while minimising the risk of development of resistance to an individual active ingredient. However, the number of effective active ingredients reduces dramatically when using drench applications or a spray programme to eradicate disease, under these circumstances resistance issues would be more of a problem.

Efficacy study – Glasshouse (large scale) (STC)

The first scheduled fungicide application was made on the 13th July, six days after the initial inoculation of plants. As there was no evidence of downy mildew expression on any of the control pansy plants by time of the second scheduled spray (20th July), even though results from the laboratory and glasshouse temperatures indicated that there should have been, the spray was postponed and monitoring for symptoms continued until 11th August. The lack of symptoms prompted crop trimming, to encourage fresh growth, and a second inoculation with *P. violae* sporangia. The trial was once again monitored for symptoms, however no symptoms developed and the trial was terminated on 8th September.

Crop safety

No evidence of true phytotoxicity symptoms was observed on either the *I. walleriana* or the *I. hawkeri* New Guinea type impatiens during this investigation. Some evidence of spray residue was evident on the plants treated with both Fubol Gold and Karamate Dryflo applied at the twice-normal (2N) rate (Figure 13), although this did gradually wash away after several irrigations.

Figure 13. *I. walleriana* showing spray residue 36 hours after the application of Karamate Dryflo.

The plants were retained for 4 weeks following the fungicide application. All treated plants budded-up and flowered normally during this period, no damage to the flowers was observed (Figure 14). As the plants had become stressed due to retention in the in the small plugs, no destructive assessments were carried out.

Figure 14. Impatiens crop safety trial plants (May 2009).

Conclusions

- Zoospore release from sporangia of *Plasmopara obducens* was demonstrated to occur between 5 and 14°C, with optimal release occurring at 10°C.
- Criteria for the infection of Impatiens by *P. obducens* have been established; overnight leaf wetness and temperatures between 5 and 10°C to encourage zoospore release from sporangia and plant infection.
- Freezing is not a viable option for the long-term storage of isolates of either *P. obducens* or *P. violae*. Maintenance will have to be through plant infection.
- A number of products with differing active ingredients and modes of action (Fubol Gold, Subdue, Bayer Exp, Syngenta Exp, Olympus, Stroby and Rose tonic) have been shown to potentially offer effective protection against the development of pansy downy mildew.
- Only products containing metalaxyl-M, and possibly Bayer Exp to a lesser extent, had any eradicant activity against pansy downy mildew infection, however, subject to approval, treatments would need to be applied soon after infection occurs.

- Olympus, Bayer Exp and Syngenta Exp (subject to approval) and possibly Stroby and Rose Tonic could all be used in alternating 7 and 14 day programmes with products containing metalaxyl-M (e.g. Subdue, Fubol Gold) to control pansy downy mildew.
- No evidence of phytotoxicity symptoms was observed on either the *I. walleriana* or the *I. hawkeri* New Guinea impatiens.

Future work

From the outset this project was intended to work on one pathogen, either *P. obducens* or *P. violae*. However the lack of *P. obducens* infections in the UK led to the decision to work on *P. violae* as a model pathogen. However, the dramatic reoccurrence of *P. obducens* in the UK during 2007 and 2008, and the difficulties encountered establishing *P. violae* infections in pansies, resulted in the project working on both pathogens. This has inevitably led to a significant increase in work load in trying to address each of the original work areas, in particular infection studies and crop safety trials, and has also resulted in some areas being uncompleted as follows:

- Detection of latent infections of impatiens downy mildew
- Determine efficacy of fungicides against impatiens downy mildew infections.
- Testing the effectiveness of potential spray programmes for the control of impatiens downy mildew.
- Production of baseline fungicide sensitivity data for *P. obducens* and *P. violae* isolates.

In addition results from the project have highlighted other areas that need addressing,

- The first detection of oospores produced by *P. obducens* in leaf and stem tissue of impatiens plants within this project, means there is the potential for contamination of soil in parks and gardens planting areas by these resting structures. Work is required to determine the length of time these structures are able to survive in soil and whether they are capable of re-infection, especially semi-mature plants in 6-packs or similar.
- Potential sources of inoculum in wild plant species need to be established, e.g. can the native Impatiens species, *I. noli-tangere*, of the introduced Indian balsam (*I. glandulifera*) become infected by *P. obducens* under UK conditions and harbour inoculum, which can infect commercially grown impatiens.

Technology transfer

- Presentation of data at the BPOA/HDC 'Disease Seminar' on the 18th February 2009 at Warwick-HRI.
- HDC Factsheet on Impatiens downy mildew (update)
- Preparation of Good Horticultural Practice guidelines for the bedding plant industry.

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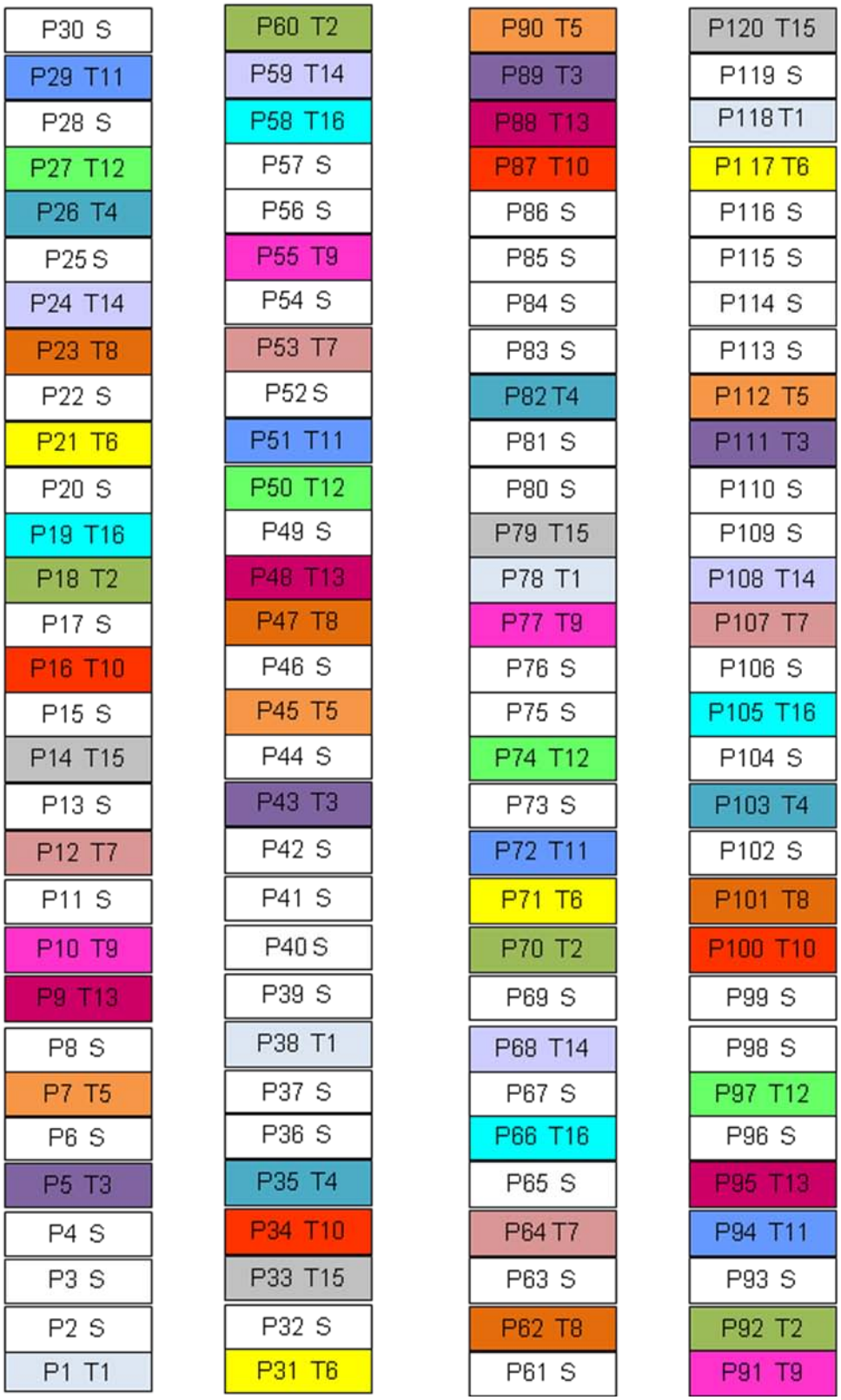
Appendix 1

Pansy – Downy Mildew Efficacy Trial E248 PC 230
July 2009



White boxes indicate additional untreated plots (S) interspersed in the randomised trial.